

Phenolics from hull of *Garcinia mangostana* fruit and their antioxidant activities

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Abstract

The air-dried fruit hulls of *Garcinia mangostana* Linn. were extracted with 70% MeOH, and then partitioned into the *n*-BuOH fractions. Furthermore, three major phenolic components related to their antioxidant activities were purified by silica gel column chromatography and Sephadex LH-20 and then identified as P₁ (1,3,6,7-tetrahydroxy-2,8-(3-methyl-2-butenyl)), P₂ [1,3,6-trihydroxy-7-methoxy-2,8-(3-methyl-2-butenyl) xanthone] and P₃ (epicatechin) using UV–visible spectrophotometry, IR spectrophotometry and NMR spectroscopy, respectively. The antioxidant activities of three major phenolics were evaluated using different tests, including the free radical scavenging capability and total antioxidant activity in a linoleic acid peroxidation. These three phenolic compounds exhibited different antioxidant activities in these antioxidant tests. The hydroxyl radical and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capabilities and the activity against linoleic acid peroxidation of P₁ were greater than those of P₂ and P₃, while the superoxide anion radical scavenging activity of P₃ was greater than that of P₁, but was close to that of P₂ or α -tocopherol. An activity-guided isolation and purification process was used to identify the components showing the strong DPPH radical scavenging activity from *G. mangostana* Linn. © 2006 Elsevier Ltd. All rights reserved.

Keywords: *Garcinia mangostana* Linn.; Phenolic; Antioxidant activity

1. Introduction

Garcinia mangostana Linn. is a climacteric fruit, named as ‘the queen of fruit’ and mostly eaten fresh. The edible aril is white, soft and juicy with a sweet, slightly acid taste and pleasant aroma. It is distributed in Thailand, India, Srilanka, Myanmar, Indonesia, Malaysia, Philippines and China. People in these countries often use *Garcinia* for traditional medicines including the treatment of abdominal pain, dysentery, diarrhoea, suppuration, infected wound, leucorrhoea and chronic ulcer and gonorrhoea (Jayaprakasha, Negi, & Jena, 2006). Furthermore, *G. mangostana* Linn. exhibits an anti-inflammatory (Gopalakrishnan, Banumathi, & Suresh, 1997), antitumor and antioxidant abilities (Williams, Ongsakul, Proudfoot, Croft, & Bellin, 1995), and antibacterial activity against *Staphylococcus aureus* (Sakagami, Inuma, Piyasena, & Dharmaratne, 2005) and *Helicobacter pylori* (Mahabusarakum, Phongpaichit, Jansakul, & Wiriyachitra, 1983). It was reported that the hull of *G. mangostana* Linn. was a source of mangostin, tannin, xanthone, isoflavone, flavone and other bioactive substances (Deachathai, Mahabusarakum, Phongpaichit, & Taylor, 2005; Jung, Su, Keller, Mehta, & Kinghorn, 2006).

The objective of this study was to extract, then purify and finally identify the major phenolic components from hull of *G. mangostana* Linn. fruit, which were related to their antioxidant activities.

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2. Materials and methods

2.1. Plant materials

The whole hull of *G. mangostana* Linn. fruit at the commercially mature stage was collected in summer, 2004, from the southwestern Guangdong Province and dried.

2.2. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), dihydronicotinamide dinucleotide (NADH), thiobarbituric acid (TBA) and deoxyribose were purchased from Sigma Chemical Co. (St. Louis, MO, USA), while linoleic acid, α -tocopherol, Trolox and BHA were obtained from Fluka Co. All other chemicals used were of analytical grade.

2.3. Extraction and isolation

According to the method reported by Huang, Chen, and Chen (2001), the air-dried hulls (100 g) of *G. mangostana* Linn. fruit were extracted with 70% MeOH (1:10, w/v) for 2 h at 50 °C. After filtering of the extract through Whatman No. 1 paper, the residue was re-extracted and then filtered. Filtrates were combined and concentrated using a rotary evaporator at 40 °C, and then lyophilized in vacuum. The MeOH extract (19.3 g) was dissolved in water and then partitioned with *n*-BuOH (400 ml fractions up to decoloration of the organic solvent), which yielded *n*-BuOH and aqueous fractions. The BuOH fraction (4.75 g) was chromatographed on a silica gel column (Merck silica gel 60, mesh > 230) and then eluted with solvent systems of CH₂Cl₂–Me₂CO (10:1) and CH₂Cl₂–MeOH (10:1), respectively. Fractions (50 ml) were collected and subjected to TLC analysis. TLC analysis was performed on silica gel (Merck, Germany, Kieselgel 60 F254, 0.2 mm thick) using *n*-hexane–CHCl₃ as the mobile phase. Compounds were visualized under UV light (λ 254 and 365 nm) or by spraying the plates with 10% (H₂SO₄ in EtOH). The former obtained 1–17 fractions, while the latter obtained 18–26 fractions after TLC analysis. Fractions 5–7 (1.02 g) from eluted previously with CH₂Cl₂–Me₂CO (10:1) was rechromatographed over a silica gel column, followed by elution with *n*-hexanes–EtOAc (5:1 v/v) to give P₂ (54 mg). Fractions 14–17 (1.73 g) were rechromatographed over a silica gel column using hexane–CHCl₃ as an eluent. Sub-fraction 1 and 2 were obtained after elution with 1:3 and 7:3 of *n*-hexane–CHCl₃, respectively. Sub-fraction 2 (0.77 g) was further subjected to Sephadex LH-20 and eluted with MeOH–H₂O (3:1) to yield P₁ (62 mg). Moreover, fractions 22–23 (0.74 g) eluted previously with CH₂Cl₂–MeOH (10:1) was further separated by a silica gel column, followed by eluted with CH₂Cl₂–MeOH (20:1) to yield two fractions (A and B). Fraction A (0.47 g) was rechromatographed on Sephadex LH-20 and eluted with MeOH–H₂O (3:1) to obtain P₃ (30 mg). The three components

(P₁, P₂ and P₃) were collected and then freeze-dried, separately.

2.4. Determination of melting point

Melting points were recorded with a digital electrothermal melting point apparatus (Electrothermal 9100, Electrothermal Engineering Ltd., Essex, UK).

2.5. UV–visible spectrophotometric analysis

Each of three major fractions (1 mg) was dissolved in 10 ml of ethanol. The sample solution was scanned from 200 to 600 nm, using a UV–visible spectrophotometer (Shimadzu UZ-2201, Shimadzu Co., Kyoto, Japan).

2.6. IR spectrophotometric analysis

The analysis was done by the method of Yang et al. (2006) with a minor modification. Each of three major fractions was mixed with KBr, while infrared spectra (IR) were obtained with a RFX-6 A spectrometer (Analect Instruments Inc., Irvine, CA, USA).

2.7. Molecular weight estimation

Mass spectrum (MS) system (LCQDECA, Finigan Inc., CA, USA), equipped with a Hewlett-Packard 9000 computer system, was used to determine the molecular weight. Sample (1 mg) was dissolved in 10 ml of ethanol. A 100 μ l of sample solution was injected into the MS system. Mass spectroscopy was recorded with a heat capillary voltage of 4.5 kV, a heat capillary temperature of 270 °C, sheath gas flow rate of 70 units and auxiliary gas flow rate of 10 units. The scan range of *m/z* was 200–1400.

2.8. NMR spectroscopy

¹H and ¹³C nuclear magnetic resonance spectra were recorded with a FT-NMR Bruker advance spectrometer (Bruker ARX400, Bruker Biospin Co., Karlsruhe, German). Samples (10 mg) were dissolved in 0.5 ml of acetone-*d*₆. ¹³C chemical shifts were expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard.

2.9. Determination of antioxidant capability

2.9.1. Assay of DPPH scavenging activity

The DPPH free radical scavenging activity was determined by the method of Shimada, Fujikawa, Yahara, and Nakamura (1992) with some modifications. Each sample at 20 μ g/ml in methanol (4 ml) was mixed with 1 ml of methanolic solution containing 1 mM DPPH. The mixture was shaken vigorously, and then left to stand for 30 min in the dark. The absorbance was measured at 517 nm. The inhibition of DPPH radicals by the samples was calculated

as follows: DPPH inhibition (%) = $[1 - \text{absorbance of sample} / \text{absorbance of control (0 } \mu\text{g/ml)}] \times 100$. α -Tocopherol, Trolox and BHA were used for controls, respectively. All the tests were performed in triplicate.

2.9.2. Determination of hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was carried out as described by Ghiselli, Nardini, Baldi, and Scaccini (1998). A sample solution (0.1 ml) at 20 $\mu\text{g/ml}$ was mixed with 0.8 ml of reaction buffer [0.2 M phosphate buffer (pH 7.4), 1.75 μmol deoxyribose, 0.1 μmol iron ammonium sulphate and 0.1 μmol EDTA]. Ascorbic acid (1.0 mM, 0.1 ml) and H_2O_2 (0.1 ml, 0.01 M) was then added to the reaction solution. The reaction solution was incubated for 10 min at 37 °C prior to the addition of 0.5 ml of 1% thiobarbituric acid and 1 ml of 2.8% trichloroacetic acid. The mixture was boiled for 10 min and cooled rapidly. The absorbance of the mixture was measured at 532 nm. Percent inhibition of deoxyribose degradation was calculated as $(1 - \text{absorbance of sample} / \text{absorbance of control}) \times 100$. α -Tocopherol and Trolox and BHA were used for controls, respectively. All the tests were performed in triplicate.

2.9.3. Assay of superoxide anion scavenging activity

The superoxide anion scavenging activity was measured by the method of Robak and Gryglewski (1988) with a minor modification. The reaction mixture contained 1 ml of 0.1 M phosphate buffer (pH 7.4), 150 μM NBT, 60 μM PMS and 468 μM NADH. After 5 min of incubation at 25 °C, the absorbance was measured at 560 nm. The percentage inhibition of superoxide anion generation was calculated using the following formula: Inhibition (%) = $(1 - \text{absorbance of sample} / \text{absorbance of control}) \times 100$. α -Tocopherol, Trolox and BHA were used for controls, respectively. All the tests were performed in triplicate.

2.9.4. Determination of antioxidant activity with the ferric thiocyanate (FTC) method

The antioxidant activity of the samples on inhibition of linoleic acid peroxidation was determined by the thiocyanate method (Mitsuda, Yasumodo, & Iwami, 1996). A methanol solution (0.5 ml) of the samples was mixed with 2.5 ml of 0.02 M linoleic acid emulsion in phosphate buffer (pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier and 50 ml phosphate buffer, and then the mixture was homogenized. The reaction mixture was incubated at 37 °C to accelerate the oxidation process. The levels of oxidation were determined by measuring the absorbance at 500 nm on a Hitachi U-2000 spectrophotometer following reaction with ferrous chloride and ammonium thiocyanate. The antioxidant activities of BHA and α -tocopherol were also assayed, respectively, at the same concentration for comparison purposes. The percent inhibition of lipid peroxidation of linoleic acid emulsion was calculated as inhibition of lipid peroxidation (%) = $100 - [(A_1/A_0) \times 100]$, where

A_0 is the absorbance of the control (Sample with 0 $\mu\text{g/ml}$) reaction, and A_1 is the absorbance in the presence of the samples.

2.10. Statistical analysis

All the data obtained were expressed as means \pm standard deviations of three replicated determinations.

3. Results and discussion

3.1. Identification of major active components

P_1 , obtained as a yellow amorphous material, had 204–206 °C of melting point. Its molecular ion of m/z $[M]^+$ 395.1 was in agreement with the molecular formula $\text{C}_{23}\text{H}_{24}\text{O}_6$. The UV spectra showed an absorption band at λ_{max} (207, 243, 259 and 318 nm), which was consistent with the absorptions of xanthone derivative. The IR spectra indicated the presence of O–H (3419 cm^{-1}) and C=O (1614 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) showed one chelated phenolic hydroxyl group [δ 13.92 (s)], two aromatic singlets [δ 6.82 (1H, s, H-4) and 6.38 (1H, s, H-5)], in addition to three hydroxyl group [9.60, 9.48, 9.38 (1H, each, br s)]. Characteristic signals of two prenyl unit were exhibited [δ_{H} 5.31 (1H, mt, $J = 6.0$ Hz), 3.36 (2H, d, $J = 6.0$ Hz), 1.80, 1.85, 1.66 and 1.66 (each 3H, s), 4.20 (1H dd, $J = 18, 10$ Hz) 3.35 (2H, d, $J = 6.0$ Hz)]. The ^{13}C NMR spectra (Table 1) showed resonances for all 23 carbons presenting in the molecule, which indicated the presence of four hydroxylated at δ 160.2, 162.0, 155.0 and 140.7 ppm, and the presence of a chelated carbonyl [δ_{C} 182.2 (s)]. The C-1 signal was found at δ_{C} 160.2 ppm, while the C-2, C-3, C-4 and C-4a signals appeared at δ_{C} 109.9, 162.0, 91.7 and 151.9 ppm, respectively. The latter signals coincided with those in ring A of a 1,3,7-trihydroxy-2,4-bis(3-methyl-2-butenyl) xanthone, had slight difference (Linuma, Tosa, Tanaka, & Yonemori, 1994) and those of C-5, C-6, C-7 and C-8 were observed at δ_{C} 99.7, 155.0, 140.7 and 130.4 ppm, respectively. According to literatures published previously (Deachathai et al., 2006; Vatcharin, Thunwadee, Athipol, Prakart, & Walter, 2003), H-4 showed correlations to C-3 (δ_{C} 162.0 ppm), C-2 (δ_{C} 109.9 ppm) and C-4a (δ_{C} 151.9 ppm). The prenyl unit was assigned to be at C-2 (δ_{C} 109.9 ppm) and C-8 (δ_{C} 130.4 ppm) by the correlation between H-16 (δ_{H} 3.38 ppm) and C-1 (δ_{C} 160.2 ppm), C-2 (δ_{C} 109.9 ppm) and C-3 (δ_{C} 162.0 ppm), and H-11 (δ_{H} 4.20 ppm) and C-7 (δ_{C} 140.7 ppm), C-8 (δ_{C} 130.4 ppm) and C-8a (δ_{C} 111.0 ppm). Since no other aromatic protons were observed, C-3 and C-6 substituents were hydroxyl groups. Therefore, P_1 was assigned as 1,3,6,7-tetrahydroxy-2,8-(3-methyl-2-butenyl) xanthone (Fig. 1).

P_2 , obtained as a yellow amorphous material, with 180–181 °C of melting point, had its molecular ion of m/z $[M]^+$ 409, which was in agreement with the molecular formula $\text{C}_{24}\text{H}_{26}\text{O}_6$. The UV spectrum showed an absorption band

Table 1
¹H NMR and ¹³C NMR data of compounds (P₁ and P₂)

C-position	P ₁		P ₂	
	¹³ C NMR	¹ H NMR	¹³ C NMR	¹ H NMR
1	160.2	13.92 (1H, s)	160.3	13.79 (1H, s)
2	109.9		110.2	
3	162.0	9.48 (1H, s)	162.3	9.48 (1H, s)
4	91.7	6.38 (1H, s)	91.9	6.41 (1H, s)
4a	151.9		154.9	
5	99.7	6.83 (1H, s)	101.6	6.83 (1H, s)
6	155.0	9.60 (1H, s)	156.6	9.59 (1H, s)
7	140.7	9.38 (1H, s)	143.5	
8	130.4		137.2	
8a	110.0		111.0	
9	182.2		181.8	
9a	102.7		102.6	
10a	152.7		155.4	
11	21.2	4.20 (1H, dd, <i>J</i> = 18, 10 Hz)	21.2	4.16 (1H, dd, <i>J</i> = 18, 10 Hz)
12	122.7	5.31 (1H, mt, <i>J</i> = 6.0 Hz)	122.7	5.28 (1H, mt, <i>J</i> = 6.0 Hz)
13	130.3		130.6	
14	25.5	1.85 (3H, s)	26.0	1.85 (3H, s)
15	16.8	1.80 (3H, s)	16.9	1.80 (3H, s)
16	25.0	3.36 (2H, d, <i>J</i> = 6.0 Hz)	25.0	3.38 (2H, d, <i>J</i> = 6.0 Hz)
17	123.6	5.31 (1H, mt, <i>J</i> = 6.0 Hz)	123.9	5.28 (1H, mt, <i>J</i> = 6.0 Hz)
18	128.3		130.4	
19	25.0	1.66 (3H, s)	25.0	1.67 (3H, s)
20	17.3	1.66 (3H, s)	17.3	1.65 (3H, s)
21			60.2	3.81 (3H, s)

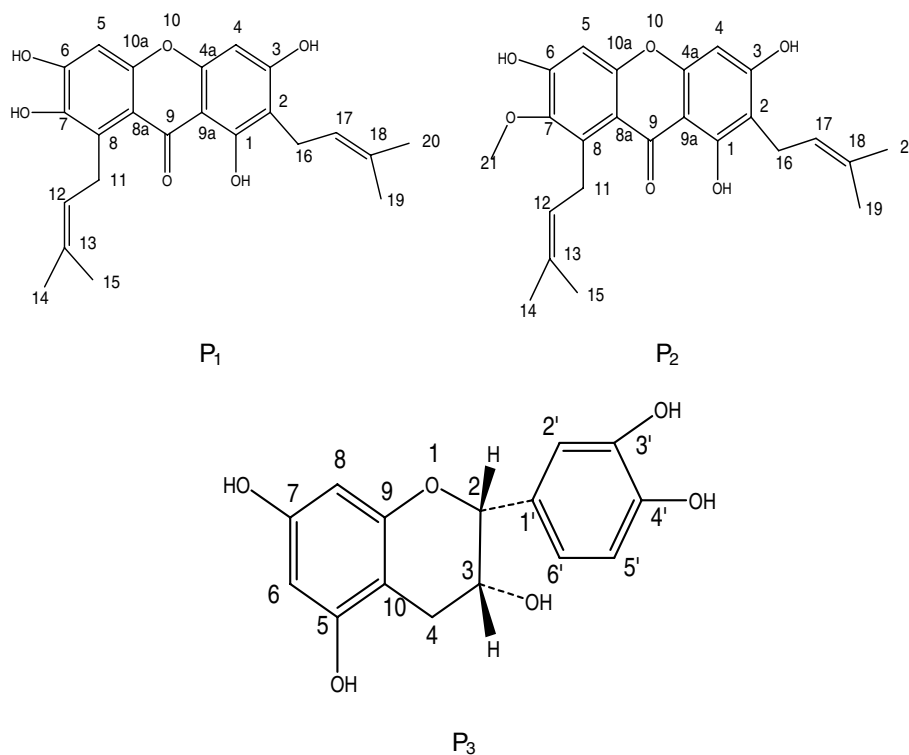


Fig. 1. Molecular formula of P₁ [1,3,6,7-tetrahydroxy-3-methoxy-2,8-(3-methyl-2-butenyl) xanthone], P₂ [1,3,6-trihydroxy-3-methoxy-2,8-(3-methyl-2-butenyl)xanthone] and P₃ (epicatechin).

at λ_{\max} (205, 243 and 317 nm) which was consistent with the absorptions of xanthone derivative. The IR spectra showed the presence of O–H (3417 cm^{-1}) and C=O

(1609 cm^{-1}) groups. Its UV and IR spectra were indicative of a xanthone derivative. It was reported by Chaudhuri, Zymalkowski, and Fram (1978) that the δ value for a meth-

oxyl carbon surrounded by two ortho substituents (OMe, O-aryl or CO-aryl) in the ^{13}C NMR spectrum of a poly-methoxy xanthone is shifted downfield to δ 60–62 ppm. The appearance of downfield methoxyl signals at δ 60.2 ppm indicated the presence of a methoxy groups. As the spectrum shown in Table 1, the ^1H and ^{13}C NMR spectral data of P_2 closely resembled those of xanthone (P_1) except for the presence in xanthone (P_2) of a methoxyl instead of a hydroxyl group. Therefore, P_2 could be assigned as 1,3,6-trihydroxy-7-methoxyl-2,8-(3-methyl-2-butenyl) xanthone (Fig. 1).

P_3 was obtained as a blank amorphous material. Its molecular formula of $\text{C}_{15}\text{H}_{14}\text{O}_6$ was established on the basis of the mass spectrum ($[\text{M}]^+ m/z$ 289). The UV spectrum showed the maximal absorption at 278 nm. In the ^1H NMR spectrum, these signals [6.01 (1H, d, $J = 2.1$ Hz), 5.86 (1H, d, $J = 2.1$ Hz)] and chelated constant ($J = 2.1$ Hz) indicated the two aromatic singlets as metaposition coupling of chromene ring (Table 2). The signals in the aromatic region, 6.88 (1H, d, $J = 1.8$ Hz), 6.76 (1H, d, $J = 8.1$ Hz), 6.72 (1H, dd, $J = 1.8$ and 8.1 Hz) appeared as an ABC type, were proposed for the signals of H-2', H-5' and H-6', respectively, which suggest the presence of two chromene ring. According to previous report (Huang et al., 2001), 2.53 (1H, ax, dd, $J = 15.9$ and 8.4 Hz) and 2.88 (1H, eq, dd, $J = 15.9$ and 5.1 Hz) exhibited the existence of a CH_2 group and CH [4.56 (1H, d,

$J = 7.5$ Hz) and 3.99 (1H, m)]. Therefore, identification of P_3 as epicatechin was apparent from the ^{13}C NMR and ^1H NMR spectra. A methylene carbon in the upfield region (δ 29.9 ppm) and two oxygenated methine carbons in the heterocyclic region (δ 67.7 and 80.3 ppm) were characteristic of the pyran C-ring of flavanols, while the upfield position of the C-2 carbon (δ 80.3 ppm) was characteristic of the epicatechin chemical shift (Lu & Foo, 1997). Thus, P_3 was identified as epicatechin (Fig. 1).

3.2. Antioxidant activity

3.2.1. Assay of radical scavenging activity

Phenolic compounds have exhibited a strong antioxidant capability (Bao, Cai, Sun, Wang, & Corke, 2005; Ghiselli et al., 1998; Robards & Antolovich, 1997). The antioxidant activities of P_1 , P_2 , P_3 and controls, BHA, α -tocopherol and Trolox, were shown in Table 3, respectively. In this study, the scavenging effect of P_1 , P_2 , P_3 and controls on the DPPH radicals decreased in the order of BHA, α -tocopherol, P_1 , P_3 , P_2 and trolox, which were 91.0%, 85.3%, 84.1%, 83.9%, 70.4%, 53.5% and 14.3%, respectively, at 20 $\mu\text{g}/\text{ml}$. The percent inhibition of superoxide anion radical generation at the same concentration was 51.8% (P_1), 72.% (P_2), and 73.7% (P_3), 48.9% (BHA), 73.0% (α -tocopherol) and 82.6% (Trolox). On the other hand, hydroxyl radical scavenging effect of those samples decreased in the order of P_1 , BHA, α -tocopherol, P_3 , Trolox and P_2 .

3.2.2. Assay of antioxidant activity with the ferric thiocyanate (FTC) method

The lipid inhibitory abilities of three phenolic compounds were compared with selected standard antioxidants, BHA, Trolox and α -tocopherol, by the FTC method through measuring the amount of peroxide produced during the initial stages of oxidation. Low absorbance value in the FTC method indicated a high level of antioxidant activity (Mitsuda et al., 1996). The results were shown in Table 4, the samples and standard antioxidants exhibited effective antioxidant activity. The antioxidant activity was evaluated in the methanol solvent system. At 40 $\mu\text{g}/\text{ml}$, P_1 exhibited 83.2% inhibition in the linoleic acid peroxidation system, which was significantly ($P < 0.05$) higher than α -tocopherol (78.5%) and Trolox (56.2%).

Table 2
 ^1H NMR and ^{13}C NMR data of P_3

C-position	^{13}C NMR	^1H NMR
2	80.3 CH	4.88 (1H, s)
3	67.7 CH	4.21 (1H, m)
4	29.9 CH_2	2.71 (1H, ax, dd, $J = 16.8, 3.0$ Hz) 2.85 (1H, eq, dd, $J = 16.8, 1.5$ Hz)
5	158.2	
6	96.8 CH	6.01 (1H, d, $J = 2.1$ Hz)
7	158.2	
8	96.4 CH	5.91 (1H, d, $J = 2.1$ Hz)
9	157.9	
10	100.6	
1'	133.1	
2'	116.1 CH	7.05 (1H, d, $J = 1.8$ Hz)
3'	145.9	
4'	146.0	
5'	116.3 CH	6.79 (1H, d, $J = 8.1$ Hz)
6'	120.2 CH	6.85 (1H, dd, $J = 1.8, 8.1$ Hz)

Table 3
Comparison of radical scavenging activity (%) of three phenolic compounds (P_1 , P_2 and P_3) and standard antioxidants, α -tocopherol, BHA and Trolox

Sample	Amount (μg)	DPPH (%)	Hydroxyl radical (%)	Superoxide anion (%)
P_1	20	84.1 \pm 1.3*	85.1 \pm 0.3*	51.8 \pm 1.0**
P_2	20	53.5 \pm 1.7*	49.4 \pm 0.9*	72.9 \pm 1.0*
P_3	20	70.4 \pm 0.5*	55.9 \pm 1.8*	73.7 \pm 0.6*
α -Tocopherol	20	85.3 \pm 0.8*	69.7 \pm 1.6	73.0 \pm 0.7*
Trolox	20	14.3 \pm 1.1*	50.1 \pm 0.2*	82.6 \pm 1.4*
BHA	20	91.0 \pm 1.4	70.7 \pm 0.9	48.9 \pm 1.1

Data were presented as means \pm standard deviations of three replicate measurements. * and ** represented $P < 0.01$ and $P < 0.05$ when compared with BHA, respectively.

Table 4

Total antioxidant activities (%) of three phenolic compounds (P₁, P₂ and P₃) and standard antioxidants, α -tocopherol, BHA and Trolox in linoleic acid emulsion peroxidation system as measured by the ferric thiocyanate method at 60 h

Sample ^a	Inhibition of lipid peroxidation ^b (%)
P1	82.2 ± 0.1*
P2	62.4 ± 1.8*
P3	57.2 ± 0.3*
Trolox	56.2 ± 1.3*
α -Tocopherol	78.5 ± 2.0*
BHA	90.9 ± 0.8

^a The concentration of all test samples was 40 μ g/ml.

^b Each value is mean \pm standard deviations of three replicate measurements. * represented $P < 0.01$ when compared with BHA.

Otherwise, the antioxidant activities of BHA, P₂ and P₃ were 90.9%, 62.4% and 57.2% at the same concentration, respectively.

4. Conclusion

The major three phenolic compounds showing a strong DPPH radical scavenging activity were purified from hull of *G. mangostana* Linn. by column chromatography and identified as 1,3,6,7-tetrahydroxy-2,8-(3-methyl-2-butenyl) xanthone (P₁), 1,3,6-trihydroxy-7-methoxy-2,8-(3-methyl-2-butenyl) xanthone (P₂) and epicatechin (P₃) by NMR and MS spectral analyses. P₁, P₂ and P₃ exhibited different levels of antioxidant activities in free radical scavenging and lipid peroxidation tests. The results from various antioxidant tests revealed that P₁ had significant antioxidant activity and free radical scavenging activity. Thus, free radical scavenging activity of hull of *G. mangostana* Linn. may be one of the mechanisms by which these compounds are used as foodstuff as well as traditional medicine.

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